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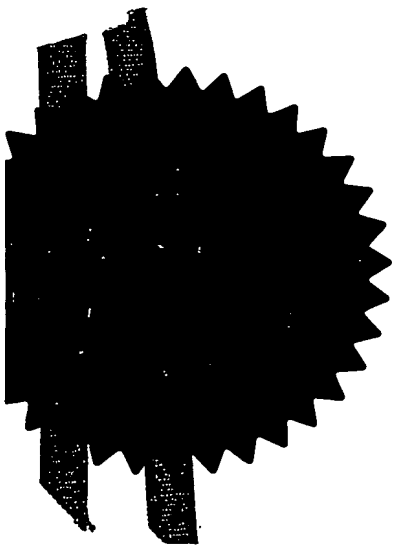

Signed Andrew Gerry

Dated 19 August 2004

Patents Form 1/77

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1/77

Request for grant of a patent

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23 SEP 2003

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1.	**Your reference**	44.95.81434/001
2.	**Patent application number** *(The Patent Office will fill in this part)*	0322279.1
3.	**Full name, address and postcode of the or of each applicant** *(underline all surnames)*	Camurus AB, Ideon, Gamma 1, Sölvegatan 41, SE-223 70 Lund, SE
	Patents ADP number *(if you know it)*	08217762002
	If the applicant is a corporate body, give country/state of incorporation	Sweden
4.	**Title of the invention**	Method
5.	**Name of your agent** *(if you have one)*	Frank B. Dehn & Co.
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	179 Queen Victoria Street London EC4V 4EL
	Patents ADP number *(if you know it)*	166001 ✓

6.	**If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**	**Country**	**Priority application number** *(if you know it)*	**Date of filing** *(day / month / year)*

7.	**If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**	**Number of earlier application**	**Date of filing** *(day / month / year)*

8.	**Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	Yes

9. **Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document**

Continuation sheets of this form	-
Description	41
Claim(s)	2
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Drawing(s)	8

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Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	-

11. **I/We request the grant of a patent on the basis of this application.**

Signature Frank B Dehn & Co **Date** 22 September 2003

12. **Name and daytime telephone number of person to contact in the United Kingdom**

Julian Cockbain
020 7206 0600

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Method

The present invention relates to methods for the production of particles suitable for the delivery of active substances. More specifically, the invention relates to methods for the production of non-lamellar amphiphile-based particles and for controlling the particle size distribution thereof.

Amphiphile-based formulations show considerable potential in the delivery of many substances, especially for *in vivo* delivery to the human or animal body. Because the amphiphile has both polar and apolar groups which cluster to form polar and apolar regions, it can effectively solubilise both polar and apolar compounds. In addition, many of the structures formed by amphiphiles/structuring agents in polar and/or apolar solvents have a very considerable area of polar/apolar boundary at which other amphiphilic compounds can be adsorbed and stabilised.

The formation of non-lamellar regions in the amphiphile/water, amphiphile/oil and amphiphile/oil/water phase diagrams is a well known phenomenon. Such phases include liquid crystalline phases such as the cubic P, cubic D, cubic G and hexagonal phases, which are fluid at the molecular level but show significant long-range order, and the L_3 phase which comprises a multiply interconnected bi-continuous network of bilayer sheets which are non-lamellar but lack the long-range order of the liquid crystalline phases. Depending upon their curvature, these phases may be described as normal (mean curvature towards the apolar region) or reversed (mean curvature towards the polar region).

The non-lamellar liquid crystalline and L_3 phases are

thermodynamically stable systems. That is to say, they are not simply a meta-stable state that will separate and/or reform into layers, lamellar phases or the like, but are the stable thermodynamic form of the mixture.

Both lamellar and non-lamellar systems have been investigated for their properties as carriers and/or excipients for dietary, cosmetic, nutritional, diagnostic and pharmaceutical agents but the non-lamellar systems are thought to have considerable advantages in terms of their high internal surface area and bicontinuous polar and apolar regions. This has led to considerable investigation of non-lamellar phases particularly in controlled-release formulations and for solubilising relatively insoluble compounds.

As discussed above, a bulk non-lamellar phase is typically a thermodynamically stable system. In addition, this bulk phase may be dispersed in a polar or non-polar solvent to form particles of a non-lamellar (especially liquid crystalline) phase in a bulk solvent. This allows the advantages of bulk non-lamellar phases to be applied in situations where use of a bulk non-miscible phase would cause problems, such as in parenteral applications. Further control of a compound's release profile may also be achieved by such a dispersion. In many cases, the liquid crystalline or L_3 phase is in thermodynamic equilibrium with the excess solvent based phase and therefore dispersions of non-lamellar particles can be prepared.

A method for the formation of dispersed particles of non-lamellar phase in solvents such as water is described in US 5,531,925. Such particles have a non-lamellar liquid crystalline or L_3 interior phase and a lamellar or L_3 surface phase and may also contain active ingredients.

Known particles of liquid crystalline or L_3 interior phase may be formed by methods such as adding to this phase a solution of surface-phase forming agent, stirring to form a coarse dispersion and fragmenting the resulting mixture.

In order to assess the presence of a liquid crystalline phase, the liquid crystalline order discussed above may be examined by use of small-angle X-ray diffraction (SAX), cryo-Transmission Electron Microscopy (cryo-TEM) or Nuclear Magnetic Resonance (NMR) spectroscopy studies. The sizes and size distributions of the dispersed particles may be examined by light scattering, particularly by use of laser light scattering instruments.

Dispersions containing active ingredients and particularly those for intravenous administration to the human or animal body are desirably colloidal, that is they should be of a particle size no greater than 10 μm, especially no greater than 5 μm and particularly no greater than 1 μm. If particles within the dispersion exceed this size then the dispersion may not be colloidally stable and there is a considerable risk of causing embolism when the preparation is administered intravenously. Furthermore, it is desirable that the distribution of particle sizes be narrow to maximise control over the release of any active agent. Where a particulate composition is to be administered by a method other than intravenously (e.g. orally, intramuscularly, subcutaneously, rectally or by inhalation), then the particle size need not be colloidal but it remains advantageous to provide a well characterised and reproducible particle size distribution in order to control the rate of decomposition of the particles and/or release of the active agents.

The particle size of a particulate composition should also be stable to storage over a considerable period of time. If the distribution of particle sizes changes significantly then the effective transport rate for composition (e.g. due to diffusion and rate of release of any active agent) may be adversely affected. Of still greater concern is the stability of particle sizes in a colloidal dispersion for intravenous administration. If the particle size distribution of such a dispersion is not stable to storage and distribution then large particles may form over time and be dangerous when administered.

In addition to control over particle size, it is desirable to maximise the proportion of particles which are in the desired, non-lamellar, phase in order to maximise the beneficial effects of this in terms of controlled release and reproducibility. The proportion of lamellar particles such as mono- or multi-lamellar vesicles should therefore be minimised.

Known methods for the formation of dispersed particles of non-lamellar phase are highly effective, but typically produce a relatively broad distribution of particle sizes and a certain proportion of "contaminant" lamellar vesicular particles. Increasing the proportion of fragmenting and/or stabilising agent (e.g. surfactant, copolymer and/or protein) in the formulation or increasing the energy input of the homogenisation process may be used to narrow the particle size distribution but at the expense of increasing the proportion of lamellar particles. There is therefore a considerable need for methods by which a dispersion of non-lamellar particles may be formed having a narrow, preferably colloidal, particle size distribution and a high proportion of non-lamellar particles.

The present inventors have now unexpectedly established that by heating lamellar and/or non-lamellar particles of appropriate composition to an elevated temperature for a short period before cooling to room temperature, the distribution of particle sizes may be narrowed, the stability of the particle size distribution improved and/or the proportion of non-lamellar particles increased.

The present invention therefore provides a method for the production of (preferably colloidal) non-lamellar particles, said method comprising forming lamellar and optionally non-lamellar particles comprising at least one structuring agent, heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide conversion of at least 50% of said lamellar particles to non-lamellar form, after cooling. This heating and cooling method may be carried out once, or as two, three, four or more sequential cycles of heating and cooling.

The present invention further provides a method for narrowing the particle size distribution (for example, as displayed by light scattering) of a sample of lamellar and/or non-lamellar particles comprising at least one structuring agent, said method comprising heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide a narrowing of said particle size distribution after cooling. This heating and cooling method may be carried out once, or as two, three, four or more sequential cycles of heating and cooling.

Because lamellar and non-lamellar particles are self-assembled systems, particles of a dispersion may collide and fuse, thereby broadening the distribution of particle sizes when the dispersion is stored. Oswald ripening may also contribute to broadening of the distribution during storage. It has, remarkably, been established that the method of heat cycling renders the distribution of particle sizes in a dispersion of lamellar and/or non-lamellar particles more stable over time.

In a further aspect, the present invention therefore provides a method for stabilising the particle size distribution (for example, as displayed by light scattering) of a sample of lamellar and/or non-lamellar particles comprising at least one structuring agent, said method comprising heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide stabilisation of said particle size distribution after cooling. This heating and cooling method may be carried out once, or as two, three, four or more sequential cycles of heating and cooling.

The heat cycling methods of the invention have surprisingly general application and appear suitable for the control of phase, particle size distribution and/or stability of many dispersed lipid formulations, especially where the thermodynamic state of the composition is non-lamellar at ambient temperature.

In a further aspect, the present invention provides non-lamellar particles comprising at least one structuring agent formed or formable by forming lamellar and optionally non-lamellar particles comprising at least one structuring agent, heating said particles to a

temperature at which conversion to non-lamellar particles takes place for a period sufficient to provide conversion of at least 50% of said lamellar particles to non-lamellar form, followed by cooling, preferably to ambient temperature. The particles may be non-colloidal (e.g. 10-200 μm), for example where the formulation is to be suitable for non-intravenous use, but are preferably colloidal.

As use herein, the term "non-lamellar" is used to indicate a normal or reversed liquid crystal phase (such as a cubic or hexagonal phase) or the L_3 phase or any combination thereof. Where a particle is described as having a non-lamellar phase or form, this indicates that at least the internal region of the particle should adopt this form. The particles will generally have two distinct regions, an internal region and a surrounding surface region. The surface region, even in a "non-lamellar" particle will often be lamellar or crystalline and may be any phase ranging from highly a ordered crystalline or liquid crystal phase to a virtually orderless fluid layer. In contrast, a "lamellar" particle, as described herein is a particle having a solvent, rather than non-lamellar, core-region.

The term "lamellar particles" is used herein to indicate vesicular particles characterised in that they comprise one or more outer lamellar bilayers of amphiphile, surrounding an inner solvent compartment.

The temperature to which the particles must be heated in order to provide the effect of the present invention will be readily established by one of skill in the art. For example, a sample of lamellar particles may be heated to a particular temperature for 4 hours and subsequently cooled to ambient temperature. The SAX scattering pattern of the sample before and after heat

treatment may then be compared and the results compared for the presence of peaks corresponding to, for example, reversed cubic or hexagonal phase. Similarly, the length of time required for conversion at any particular temperature may be assessed by heating samples for set times and examining any changes in SAX scattering. Equivalent heating experiments will also determine the effect upon particle size distribution and storage stability, using analytical tools such as light scattering and cryo transmission electron microscopy.

Typically, samples will be heated to a temperature in the range 75 to 200°C, preferably 85 to 150°C, more preferably 96 to 140°C. The most preferred temperature range is 100 to 130°C. The heat may be supplied by any appropriate method, such as by autoclaving, baking in an oven, by electromagnetic irradiation (e.g. infra-red or microwave irradiation) and/or alternatives known in the art.

It has been surprisingly established that the temperature cycling method of the present invention functions without the need for the equilibrium form of the composition to be non-lamellar at the elevated temperature. For example, a cubic phase may be the equilibrium condition for a composition at temperatures from ambient to 90°C and the elevated temperature be 100°C. At this elevated temperature, the equilibrium condition for a composition may not be non-lamellar. For example, the equilibrium condition for the composition at the elevated temperature may be lamellar, micellar (e.g. L1, L2) or isotropic.

Thus, the present invention also provides a method for the production of (preferably colloidal) non-lamellar particles, said method comprising forming lamellar and optionally non-lamellar particles comprising at least

one structuring agent, heating said particles to an elevated temperature at which temperature the equilibrium form of the particles is not non-lamellar (preferably lamellar, micellar (e.g. L1, L2), or isotropic), followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide conversion of at least 50% (by particle number) of said lamellar particles to non-lamellar form, after cooling. This heating and cooling method may be carried out once, or as two, three, four or more sequential cycles of heating and cooling.

Typical periods of heating at an elevated temperature are relatively short and will generally be between 1 minute and 4 hours, more typically between 2 minutes and 1 hour. Periods of between 2 and 30 minutes are preferred, particularly between 5 and 20 minutes. The period may optionally include a period for equilibration, typically 1-10 minutes.

The components of the formulations include at least one structuring agent (typically an amphiphile) and will generally also include a fragmentation agent (which may also be an amphiphile, such as a surfactant, copolymer and/or protein). In addition, the formulations of the invention may include protein, drug, nutrient, cosmetic, diagnostic, pharmaceutical, vitamin, or dietary agents at a level sufficient to be effective without disrupting the phase behaviour of the composition in such a way that a non-lamellar phase in no longer formed. These are referred to herein as "active agents". Under some circumstances the structuring agent or fragmentation agent may also be an active agent. It is preferable that the thermodynamic equilibrium state of the component mixture of the formulation at ambient temperature, optionally in the presence of a solvent

(such as water) is a non-lamellar phase such as the normal or reversed cubic or hexagonal phases or L_3 phase.

Where an active agent is formulated in a composition of or for use in the method of the invention, the active agent will frequently have an effect upon the phase behaviour of the structuring agent(s). For example, certain active agents (such as cyclosporin A) introduce greater negative curvature than some structuring agents and at high concentrations may cause the formation of highly negatively curved phases, such as the reversed micellar L_2 phase rather than a cubic or hexagonal liquid crystalline phase. Nonetheless, such an active agent could be formulated into, for example, a reversed hexagonal phase by formulation with a structuring agent, or a blend thereof, having a less negative spontaneous curvature. By this method, the overall mixture provides the appropriate negative curvature to allow use in the methods or compositions of the invention.

The skilled worker will be able to use standard methods to assess the degree of spontaneous curvature of any particular structuring agent (or mixture thereof) or the effect on this by including an active agent. This might be done, for example, by studies of the bulk phase behaviour of each structuring agent in water and subsequent studies with varying concentrations of active agent included. The phases can be examined by any of the methods indicated herein (e.g. polarised light, SAXS cryo-TEM etc.) and an appropriate blend of structuring agents chosen for each case. In some circumstances, where the effect of the active agent on the phase behaviour of the mixture is significant, the structuring agent(s) chosen may not provide the desired non-lamellar phase in themselves (e.g. may have too small or too great spontaneous curvature) but will generate this phase only when also formulated with the active agent.

Similarly, the equilibrium phase may change from, for example, cubic to hexagonal liquid crystalline phase upon addition of the active agent.

The term structuring agents, as used herein in the methods and compositions of the invention, are any agents that are capable of forming a non-lamellar phase, optionally in the presence of other agents such as amphiphiles and/or fragmentation agents. Structuring agents will generally have at least one polar, hydrophilic group and at least one non-polar, hydrophobic group. A wide range of structuring agents are applicable for use as all or part of the structuring agent component.

Examples of polar groups are well known (see e.g. US published patent application number 20020153509) and include anionic groups such as carboxylates, phosphonates, sulphates and sulphonates, non-ionic groups such as alcohols, polyols (eg sugars, glycerol etc) and esters, cationic groups such as quaternary ammonium compounds, pyridinium salts and quaternary phosphonium salts and zwitterionic groups such as phospholipid head groups (e.g phosphatidyl-choline, phosphatidic acid, phosphocholine, phosphoethanolamine, phosphoglycerol, phosphoserine, their PEGylated or mPEGylated derivatives, etc.), ammonioacetates, ammonio-alkanesulphonates and trialkylaminoalkylphosphate esters.

Examples of non-polar groups include C_6-C_{32} alkyl and alkenyl groups, which are typically present as the esters of long chain carboxylic acids. These are often described by reference to the number of carbon atoms and the number of unsaturations in the carbon chain. Thus, CX:Y indicates a hydrocarbon chain having X carbon atoms and Y unsaturations. Examples particularly include

caproyl (C6:0), capryloyl (C8:0), capryl (C10:0), lauroyl (C12:0), myristoyl (C14:0), palmitoyl (C16:0), phytanoly (C16:0), palmitoleoyl (C16:1), stearoyl (C18:0), oleoyl (C18:1), elaidoyl (C18:1), linoleoyl (C18:2), linolenoyl (C18:3), arachidonoyl (C20:4), behenoyl (C22:0) and lignoceroyl (C24:9) groups. An amphiphile will typically have one or two non-polar "tail" groups (mono-acyl and di-acyl lipids respectively) but may have three, four or more hydrophobic groups.

Examples of structuring agents suitable for use in the present invention include natural lipids, synthetic lipids, surfactants, copolymers, peptides, proteins, hydrotropes, alcohols, and other additives that may form or facilitate formation of non-lamellar structures. Preferred agents are glycerides (e.g. monoglycerides, diglycerides, and triglycerides), di- and polyglycerolesters of glycerides (e.g. diglycerol monooleate, diglycerol monocaprate), natural fats and oils (e.g. soybean oil, coconut oil, corn oil, castor oil, sunflower oil), fractionated oils (e.g. fractionated coconut oil, Miglyol® (Condea)), transesterified oils (e.g. Maizine®), transesterification products of oils and PEG (e.g. ethoxylated castor oil (e.g. Cremophor® EL (BASF)), ethoxylated hydrogenated castor oil (e.g. Cremophor® RH-40 (BASF)), ethoxylated corn oil (e.g. Labrafil® M 2125 CS (Gattefossé))), acetylated monoglycerides, fatty acids (e.g. C6-C26 saturated and unsaturated fatty acids), fatty alcohols (e.g. phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecantriol)), ether lipids (e.g. monooleyl glyceryl ether), natural and synthetic phospholipids (e.g. egg lecithin, soya lecithin, hydrogenated lecithin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl glycerol, phosphatidic acid),

lysophospholipids (e.g. lyso-lecithin, lyso-phosphatidyl choline, lyso-oleyl phosphatidyl choline), phospholipid-analogous compounds (e.g. those disclosed in US 6344576), sterols and sterol derivatives (e.g. cholesterol, sitosterol, lanesterol and their esters, especially with PEG or fatty acids), galactolipids (e.g. digalactosyl diacylglycerol, monogalactosyl diacylglycerol), sphingolipids (e.g. sphingomyelin); nonionic surfactants, in particular ethoxylated surfactants such as PEG-fatty acid mono- and diesters (e.g. of the Crodet® (Croda), Cithrol® (Croda), Nikkol® (Nikko), Myrj® (ICI) series, Solutol® HS 15 (BASF)), PEG glycerol fatty acid esters (e.g. Tagat® L and O (Goldschmidt), Glycerox® L series (Croda), Capmul® EMG (Abitec)), transesterification products of oils and PEG (e.g. of the Labrafil® (Gattefossé), Cremophor® (BASF) Crovol® (Croda) and Nikkol® HCO (Nikko) series) , PEG-sorbitan fatty acid esters (e.g. Tween® 20, Tween® 80 and other polysorbates of the Tween® series (ICI)), PEG alkyl esters (e.g. of the Brij® (ICI) and Volpo® (Croda) series), PEG alkyl phenol surfactants (e.g. of the Triton X and N series (Rohm & Haas); polyglycerised fatty acids (e.g. Nikkol® Decaglyn (Nikko), Plurol® Oleique (Gattefossé)), propylene glycol fatty acid esters), propylene glycol fatty acid esters (e.g. Capryol® 90 (Gattefossé), Lutrol® OP2000 (BASF), Captex® (Abitec)), glycerol/propylene glycol fatty acid esters (e.g. Arlacel® 186 (ICI)), sorbitan fatty acid esters (e.g. of the Span® (ICI) and Crill® (Croda) series), sugar esters (e.g. of the SUCRO ESTER® (Gattefossé) and Crodesta® (Croda) series), polyoxyethylene-polyoxypropylene block copolymers (so-called poloxamers, e.g. of the Pluronic® (BASF), Synperonic® (ICI) and Lutrol® (BASF) series), copolymers of ethylene oxide and butylene oxide; anionic surfactants including fatty acid salts, bile salts (e.g. sodium cholate, sodium glycocholate, sodium

taurocholate), carboxylates such as ether carboxylates, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono- and diglycerides, citric acid esters of mono- and diglycerides, glyceryl-lacto esters of fatty acids, acyl lactylates, alginate salts, propylene glycol alginate; cationic surfactants including ethoxylated amines (e.g. polyoxyethylene-15 coconut amine), betaines (e.g. N-lauryl-N,N-dimethylglycine), alkylpyridinium salts, quarternary ammonium salts such as hexadecyl triammonium bromide, decyl trimethyl ammonium bromide, cetyl trimethyl ammonium bromide; zwitterionic surfactants including trimethylammonioethylalkylphosphonates (e.g. the examples disclosed in US 6344576); and all mixtures thereof. The most preferred structuring agents are monooleate, monolinoleate, glyceryl dioleate, dioleyl phosphatidyl ethanolamine (DOPE), dioleyl phosphatidylcholine (DOPC) and phytantriol, and mixtures of these with up to 50% fatty acids, in particular oleic acid and linoleic acid, polysorbate 80 (Tween® 80), polyethylene glycol 660 12-hydroxysterate (Solutol® HS 15), or lyso-phospholipids, especially lyso-oleyl phosphatidylcholine (LOPC).

Often the structure forming agent component will contain components in the form of extracted and purified natural products and will thus contain a mixture of related compounds. Soy bean phosphatidyl choline, for example is a mixture of compounds having around 60-75% C18:2 acyl groups, around 12-16% C16:0 and the balance others. Similarly, commercial glycerol monooleate is typically at least 90% monoglyceride but contains small amounts of diglyceride and free fatty acid, with the acyl groups being over 60-90% C18:1, 5-10% saturated and the remainder largely higher unsaturated acyl groups. Different commercial preparations will also vary slightly as indicated in the Examples below.

A highly preferred structuring agent for use in the present invention is commercially available glycerol monooleate (GMO). As indicated above, this is largely monoglyceride with an oleoyl (C18:1) acyl chain but contains certain amounts of other compounds. These are included in the term "glycerol monooleate" or "GMO" as used herein. Commercial preparations of GMO include GMOrphic-80 and Myverol 18-99 (available from Eastman Kodak), Rylo MG 19 and Dimodan DGMO (available from Danisco). Any of the structuring agents may be used alone or in combination with one or more other structuring agents.

In addition to the amphiphilic structuring agent component, the compositions of the invention may, in particular, include at least one fatty acid or fatty acid salt component. Preferred fatty acids have between 6 and 24 carbons and particularly those corresponding to the fatty acid chains of natural lipids, including caproic, caprylic, capric, lauric, myristic, palmitic, phytanic, palmitolic, stearic, oleic, elaidic, linoleic, linolenic, arachidonic, behenic or lignoceric acids, their salts or mixtures thereof. The fatty acids may be saturated but are preferably unsaturated. The most preferred fatty acid is oleic acid. Salts of fatty acids will typically be physiologically tolerable, and for pharmaceutical applications will always be so. Preferred salts include alkali and alkaline earth metal salts such as sodium, potassium, lithium, calcium or magnesium salts as well as ammonium and alkylammonium salts. Typically, the fatty acid or fatty acid salt will be present as 0-10 wt% of the total amphiphilic component, preferably 3-7% by weight.

The fragmentation agent for use in the method of the invention will be at least one agent which aids the dispersal of the non-lamellar phase into particles or

stabilises such particles. Typically a fragmentation agent will be a surfactant such as an amphiphilic block copolymer. A large number of surfactants and copolymers are suitable for use as all or part of the fragmentation agent for use in the present invention.

Important fragmentation agents include natural lipids, synthetic lipids, surfactants, copolymers, proteins (in particular caseins and albumin), hydrotropes, alcohols and other additives that may facilitate fragmentation spontaneously or with the aid of externally applied forces and pressures and contribute to stabilisation. This includes also nanoparticles and combinations of polymer and nanoparticles (see e.g. WO 99/12640).

Suitable copolymers for use as fragmentation agents may have blocks comprising polyoxyalkylenes, polyvinylpyrollidone, polyvinylacetate, polyvinylalcohol, polyesters, polyamides and/or polyalkenes. The block copolymer will comprise at least two blocks of polymer having different degrees of hydrophilicity. Certain proteins (such as casein) are also of amphiphilic character and may be used as fragmentation agents. Where an active agent is an amphiphilic protein, this may act as both the active agent and the fragmentation agent, or may be included in addition to another active agent and/or fragmentation agent.

Preferred examples of amphiphilic block copolymers are poloxamers, which comprise at least one block of polyoxyethylene and at least one block of polyoxypropylene. The most preferred fragmentation agents are poloxamer 407 (e.g. Pluronic® F127, BASF), poloxamer 188 (e.g. Pluronic® F68, BASF), poloxamer 124 (Pluronic® L44, BASF), and polysorbates 20, 60 and/or 80 (e.g. Tween® 80, ICI). Other suitable surfactants

copolymers may be found in the "Handbook of Pharmaceutical Excipients" (2nd Ed., the American Pharmaceutical Association and The Pharmaceutical Press, Royal Pharmaceutical Society of Great Britain).

Other preferred fragmentation agents include polyethylene glycol lipid conjugates (e.g. PEGylated and mPEGylated phospholipids) as well as long chain alcohols and fatty acids.

The fragmentation agent will be present at a level sufficient to bring about the fragmentation of the structuring agent and/or to stabilise the fragmented non-lamellar phase particles. Such fragmentation may be spontaneous or may require physical fragmentation such as by shearing and/or ultrasonication. It is preferable that sufficient fragmentation agent is present that the non-lamellar particles are physically stable.

In one preferred embodiment, the compositions of and for use in the present invention consist of GMO and one or more poloxamers with any optional active agent and/or aqueous component. In an alternative embodiment, since the invention is applicable to a wide range of compositions, the compositions may comprise other structuring agent(s) and/or fragmentation agent(s) (e.g. other lipids, surfactants and/or fatty acids), with GMO and/or poloxamer optionally also present, along with any optional components such as active agents, aqueous components etc.

Active agents suitable for inclusion in the methods and formulations of the present invention include human and veterinary drugs and vaccines, diagnostic agents, "alternative" active agents such as plant essential oils, extracts or aromas, cosmetic agents, nutrients, dietary supplements etc. Examples of suitable drugs

include antibacterial agents such a β-lactams or macrocyclic peptide antibiotics, anti fungal agents such as polyene macrolides (e.g amphotericin B) or azole antifungals, anticancer and/or anti viral drugs such as nucleoside analogues, paclitaxel and derivatives thereof, anti inflammatorys, such as non-steroidal anti inflammatory drugs, cardiovascular drugs including cholesterol lowering and blood-pressure lowing agents, analgesics, antidepressants including serotonin uptake inhibitors, vaccines and bone modulators. Diagnostic agents include radionuclide labelled compounds and contrast agents including X-ray, ultrasound and MRI contrast enhancing agents. Nutrients include vitamins, coenzymes, dietary supplements etc. The active agents for use in the present invention will generally not be poloxamers or acylglycerols.

Preferred active agents include human and veterinary drugs selected from the group consisting of peptides such as adrenocorticotropic hormone (ACTH) and its fragments, angiotensin and its related peptides, antibodies and their fragments, antigens and their fragments, atrial natriuretic peptides, bioadhesive peptides, Bradykinins and their related peptides, calcitonins and their related peptides, cell surface receptor protein fragments, chemotactic peptides, cyclosporins, cytokines, Dynorphins and their related peptides, endorphins and P-lidotropin fragments, enkephalin and their related proteins, enzyme inhibitors, fibronectin fragments and their related peptides, gastrointestinal peptides, growth hormone releasing peptides, immunostimulating peptides, insulins and insulin-like growth factors, interleukins, luthenizing hormone releasing hormones (LHRH) and their related peptides, melanocyte stimulating hormones and their related peptides, nuclear localization signal related peptides, neurotensins and their related

peptides, neurotransmitter peptides, opioid peptides, oxytocins, vasopressins and their related peptides, parathyroid hormone and its fragments, protein kinases and their related peptides, somatostatins and their related peptides, substance P and its related peptides, transforming growth factors (TGF) and their related peptides, tumour necrosis factor fragments, toxins and toxoids and functional peptides such as anticancer peptides including angiostatins, antihypertension peptides, anti-blood clotting peptides, and antimicrobial peptides; selected from the group consisting of proteins such as immunoglobulins, angiogenins, bone morphogenic proteins, chemokines, colony stimulating factors (CSF), cytokines, growth factors, interferons, interleukins, leptins, leukemia inhibitory factors, stem cell factors, transforming growth factors and tumor necrosis factors; selected from the group consisting of antivirals, steroidal antiinflammatory drugs (SAID), non-steroidal anti-inflammatory drugs (NSAID), antibiotics, antifungals, antivirals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants, cardiovascular drugs including lipid lowering agents and blood-pressure lowering agents, bone modulators; vaccines, vaccine adjuvants, immunoglobulins and antisera; diagnostic agents; cosmetic agents, sunscreens and self-tanning agents; nutrients; dietary supplements; herbicides, pesticides, and repellents. Further examples of active agents can be found for instance in Martindale, The Extra Pharmacopoeia.

In the methods of the invention, particles comprising a structuring agent are formed prior to one or more heat treatment cycles. This pre-formulation will typically be in the form of a dispersion and may be prepared by established methods, such as those indicated in the present Examples and in US 5,531,925, WO 02/02716, WO 02/068561, WO 02/066014 and WO 02/068562. The disclosures of these and all references cited herein are hereby incorporated herein by reference. Such methods include adding an amphiphile/water liquid crystal phase to an aqueous solution of fragmentation agent and optionally a lipid (such as PC) and either allowing natural fragmentation of the mixture or accelerating the process with, for example, mechanical agitation, vortexing, roto-stator mixing, high-pressure homogenization, microfluidisation and/or ultrasound.

Since the method of the present invention can be used to convert lamellar particles to non-lamellar form, it is not essential that the pre-preparation particles be non-lamellar. They should, preferably, be formulated such that the thermodynamically stable state at ambient temperature is non-lamellar. Where present, the active agent may be incorporated into the particles prior to and/or after heat cycling. Where more than one heat cycle is used, the active agent may also or alternatively be incorporated between cycles. Where the active agent is heat sensitive (e.g. peptide or protein) the active agent is preferably incorporated after heat cycling is complete.

Prior to, and/or after heat-cycling, the particles may be concentrated (e.g. by ultrafiltration or dialysis) and/or dried, for example by spray drying, fluid bed drying or freeze drying. In the case of dried particles, the drying process may be followed by particle size enlargement through single or repeated

agglomeration and granulation steps. The concentrated, dried and/or agglomerated particle formulations thus formed may be used as such or hydrated and/or dispersed to yield non-lamellar particle dispersions suitable for use in the delivery of active substances, especially *in vivo*. Such concentrated, dried and/or agglomerated particle formulations and the dispersions resulting from their re-suspension/hydration form a further aspect of the present invention.

In a preferred aspect of the invention, an initial pre-formulation, prior to heat treatment, is formed in which the particles will preferably be small colloidal sized particles, for example in the range 0.02 to 0.2 μm. Preferably the mean particle size for the small colloidal particles will be 0.05 to 0.15 μm in this pre-formulation. This small particle size can be achieved by known methods, as discussed above, but such methods result in a relatively large proportion of lamellar phase particles. At least one heat treatment cycle may then be applied to the pre-formulation so as to both convert the bulk of the lamellar particles to non-lamellar form and preferably also to narrow the particle size distribution. In this process, the mean particle size typically increases but the distribution of particle sizes is reduced. In this method, at least 50% (by particle number) of the lamellar particles should be converted to non-lamellar form. Preferably, at least 75% of the lamellar particles will be converted, more preferably at least 85% (e.g. 90%). It is most preferable that the treatment method convert 99% or more of the lamellar particles to a non-lamellar form.

The presence of particles in non-lamellar form will preferably be assessed from a set of cryo-transmission electron microscopy particle images. Such images will typically show at least 30 particles, preferably they

will show a sample of more than 50 and most preferably more than 100 particles. Example images are shown in Figures 3 and 4. The presence of non-lamellar particles may also be assessed by X-ray scattering experiments.

After treatment with one or more heating and cooling cycles, the final particles should be in the colloidal size range. These will typically have an average (mode) particle size in the range 0.2 to 0.8 μm, more preferably 0.3 to 0.6 μm. It is particularly important that preparations for use in intravenous administration should not contain particles in the non-colloidal range (e.g >5 μm, especially >10 μm, as indicated herein). This may be achieved by using the method of the invention, beginning with small colloidal particles as described above. Alternatively, or in addition, the particles, preferably after heat cycling, may be filtered in order to remove non-colloidal particles.

The samples of particles formed by the present invention have a greater proportion of non-lamellar particles, a narrower distribution of (especially colloidal) particle sizes and/or greater particle size stability than has been achieved by previous methods. Such particles therefore form a further aspect of the invention, as do dispersions thereof. The particles formed or formable by the method of the invention may be used in the production of nutritional, dietary, cosmetic, diagnostic veterinary or pharmaceutical compositions by known methods using well known carriers, excipients and other ingredients. In the case of pharmaceutical compositions, the particles will be formulated with at least one pharmaceutically acceptable carrier or excipient and may be formed into tablets, capsules and so forth. The particles may also be formulated as a pre-prepared dispersion in an acceptable liquid, such as water, or dried (e.g. spray dried or freeze dried) and

sealed in sterile containers for re-suspension prior to administration.

In the formulations formed or formable by the method of the present invention, at least 75% (by volume) of the particles will preferably be non-lamellar. More preferably, at least 85% and most preferably at least 95% of particles in the formulation will be non-lamellar, as measured by volume. This measurement may be made by, for example, laser diffraction, preferably combined with cryo-TEM or SAXS (to confirm the non-lamellar particle structure) following laser diffraction.

In a further aspect, the present invention thus provides a formulation of (preferably colloidal) particles comprising at least one structuring agent, wherein at least 75% of the particles, preferably at least 85% and most preferably at least 95% of particles (as measured by volume) in the formulation are non-lamellar (e.g. as judged by laser diffraction combined with cryo-TEM or SAXS). In colloidal formulations, the average (mode) particle size will typically be in the range 0.3 to 0.6 μm, for example as determined by light scattering methods (e.g. laser diffraction). Preferably, no more than 1% of particles will be outside the range 0.05 to 1.5 μm, more preferably, not more than 0.1% will be outside this range, and most preferably no detectable (by laser diffraction) proportion of particles will be outside this range. In non-colloidal formulations the average (mode) particle size will typically be in the range 10 to 200 μm.

Furthermore, the colloidal formulations prepared by the method of the present invention are physically stable to storage over extended periods at ambient temperature. Such formulations should be essentially stable both in

terms of phase behaviour and particle size for periods of at least 10 days at room temperature, more typically at least 3 months, preferably at least 6 months and more preferably 12 months or more. In contrast, even dispersions of similar mode particle size which have not undergone treatment in the method of the invention may have particle sizes stable for less than 10 days at room temperature.

A particle size distribution can be considered essentially stable to storage if the mode particle size increases no more than two fold during the storage period. Preferably, the mode size should increase no more than 50% and more preferably no more than 20% during the storage period. Similarly, the width of the distribution at half-height should preferably increase by no more than 50%, more preferably by no more than 20% and most preferably no more than 10% during the storage period. Where a distribution is monomodal, it should preferably remain monomodal during the storage period. In a highly preferred embodiment, the distribution of sizes of particles of the compositions formed or formable by the methods of the invention alter in mode particle size and particle size distribution width at half-height by no more than 10% and remain monomodal on storage for the periods indicated above.

It is particularly important in the case of colloidal dispersions for use in intravenous or intra-arterial administration that the particle size distribution be stable to storage. A composition containing even a relatively small component of non-colloidal particles may cause embolism, or at least unpredictable rates of release upon administration directly to the blood stream. Similarly, the controlled release of an active agent may be dependent upon a reliable particle size distribution in a composition for administration by any

other route. Pharmaceutical, diagnostic and veterinary products are also desirably stable to storage for several months or the cost and availability of the product is significantly adversely affected. The method of the invention thus significantly improves the prospect of an active agent formulated in a dispersion of non-lamellar particles forming a safe and available product.

The invention will be illustrated below by the following non-limiting examples and the accompanying figures in which:

Figure 1 shows the particle size distribution of a sample of GMO with 12% poloxamer before and after heat treatment;

Figure 2 shows the particle size distribution of a sample of GMO with 8% poloxamer before and after heat treatment;

Figure 3 shows a cryo-transmission electron micrograph of a sample without heat treatment;

Figure 4 shows a cryo-transmission electron micrograph of a sample after heat treatment;

Figure 5 shows the particle size of a sample before and after heat treatment for various periods;

Figure 6 shows the particle size distribution of samples before and after heating to 80°C and 121°C;

Figure 7 shows the particle size distribution of a sample before and after heat treatment at various temperatures;

Figure 8 shows the effect of heat treatment at varying poloxamer concentrations;

Figure 9 shows the effect of heat treatment of compositions containing two different poloxamer types;

Figure 10 shows small angle X-ray scattering (SAXS) patterns for two samples, containing two different poloxamer types, after heat treatment;

Figure 11 shows the effect of storage on the SAXS for samples with and without heat treatment (curves after 20 days and 6 months are not on the same scale);

Figure 12 shows the comparative effect of heat treatment on the particle size distribution of a liposomal sample;

Figure 13 shows the comparative effect of heat treatment on the SAX pattern of a liposomal sample;

Figure 14 shows the particle size distribution of a composition of GMO, poloxamer and oleic acid before and after heat cycling;

Figure 16 shows the particle size distribution of a further composition of GMO, poloxamer and oleic acid before and after heat cycling; and

Figure 16 shows the particle size distribution of a composition of GMO, poloxamer and oleic acid with and without heat cycling after 11 days' storage.

Examples:

The materials used in the following examples were as follows:
GMOrphic-80 (Eastman Kodak)

Myverol 18-99 (Eastman Kodak),
Rylo MG 19 (Danisco)
Dimodan DGMO (Danisco)
poloxamer 407 (Pluronic® F127, BASF)
poloxamer 188 (Pluronic® F68, BASF)
polysorbate 80 (Tween® 80, ICI)

Approximate compositions of the batches used are shown below in Table 1

Table 1

	Composition %				
Trade Name	Mono-glyceride	Di-glyceride	C18:1	Saturated	Higher unsaturated
GMOrphic-80 Lot No. D0116-1293 Batch No. 1997014177	≥ 94.0	?	≥ 75	≤ 10.0	≤ 15.0
Myverol 18-99 Batch No. 1996013291	≥ 90	?	60-65	5-7	ca. 30
Dimodan DGMO, NF Lot No. 70201	98	1.5	80	7.1	11.4
Rylo MG 19, NF Lot No. 2119/53	98.7	1.0	90.3	4.7	6.6

In the following examples the abbreviations used are:

LD	Laser Diffraction particle size measurement
LM	Light microscopy
LS	Light Scattering particle size measurement
P407	poloxamer 407
P188	poloxamer 188
PCS	Photon Correlation Spectroscopy
PIDS	Polarisation Intensity Differential Scattering
PSD	Particle Size Distribution
SAXS	Small Angle X-ray Scattering

TEM Transmission Electron Microscopy

Example 1 - forming a pre-formulation

A coarse dispersion of largely cubic particles was formed by melting GMOrphic-80 (1.84 g) with poloxamer 407 (0.16 g) and adding 1.25 g of the molten mixture dropwise to deionised water (23.75 g) (containing 0.01% thiomersal as preservative) under stirring at room temperature. The resulting coarse dispersion was allowed to equilibrate for at least about 1 day before homogenisation in a microfuiudizer at high pressure (350 bar) for 15 min at 40°C.

All of the dispersions used in the following Examples were prepared according to this standard procedure (Microfluidizer, 40°C, 350 bar, 15 min) with variations in composition (poloxamer/monoolein content and poloxamer/monoolein type) as specified. Where no specific poloxamer is indicated, poloxamer 407 was used.

Typical examples of the compositions prepared by this method are:

"8% P407":	Monoolein:	1.15 g	4.6 %
	Poloxamer 407:	0.10 g	0.4 %
	Water:	23.75 g	95.0 %
"12% P407":	Monoolein:	1.10 g	4.4 %
	Poloxamer 407:	0.15 g	0.6 %
	Water:	23.75 g	95.0 %
"8.75% P188":	Monoolein:	1.1406 g	4.6 %
	Poloxamer 188:	0.1094 g	0.4 %
	Water:	23.75 g	95.0 %

Example 2- Phase analysis of dispersion without heat treatment

A dispersion was prepared with Rylo MG19 and 12% poloxamer 407 (referring to the sum of monoolein and poloxamer). The resulting system was a slightly translucent homogenous dispersion, had particle sizes mainly around 0.09 μm (plus small amounts of particles around 0.3 μm) and displayed only extremely weak, unassignable SAXS reflections. By Cryo-TEM, mainly small, lamellar particles were observed with a small proportion of non-lamellar particles (see Fig. 3). The smallest particles were all lamellar, but of the larger particles some displayed internal structure (possibly cubic) and some did not.

Example 3 - Effect of Heat Treatment

A freshly prepared dispersion containing Rylo MG19 as monoolein and 12% poloxamer P407 was divided into two fractions. One fraction was autoclaved (121°C, 15 min (plus an equilibration time of 5 min, noted in the following as "(+5 min)", if applied)) and compared to the non-autoclaved fraction. The non-autoclaved fraction was comparable to Example 2, i.e. an opaque homogenous dispersion with particle sizes mainly around 0.09 μm (plus a small number of particles around 0.3 μm) (Fig. 1) and no SAXS reflections. The heat-treated fraction was milky-white (non-transparent) and LS+PIDS analysis (Fig. 1) gave a narrow monomodal particle size distribution (around 0.27 μm, without a smaller particle size fraction).

Clear SAXS reflections could be observed for the heat treated sample indicating the presence of cubic P phase.

This indicates that the small non-cubic particles in the 0.1 μm range form larger, cubic particles in the medium sized range (ca. 0.3 μm) during the autoclaving process.

Cryo-TEM was performed on autoclaved fraction and compared to Example 2. Only a few small non-cubic particles could be detected after heat treatment. Most of the detectable particles are cubic and in the range of ca. 200-300 nm (Fig. 4). This result is in agreement with the SAXS- and LD+PIDS results of these dispersions: no cubic reflections and a particle size maximum at ca. 0.09 μm in the case of the non- autoclaved dispersion, reflections according to cubic phase type P and a particle size maximum at ca. 0.27 μm in the case of the autoclaved dispersion.

Similar behaviour was observed for a dispersion containing 8% poloxamer. In this case, the non-autoclaved dispersion is already milky white and displays SAXS reflections (cubic P); the main particle size is in the range of 0.5 μm besides lesser amounts in the range of 0.1 μm and 1.5 μm. Like in the dispersion with 12% poloxamer, aggregates become observable by LM after autoclaving, the small particles vanished and the amount of particles in the medium range increased in LD+PIDS analysis (Fig. 2).

Example 4 - Effect of Filtration

Four dispersions were prepared with 12% poloxamer, two of them with GMOrphic-80, the others with Rylo MG 19. In the case of GMOrphic, high pressure homogenization also led to opaque dispersions, similar to previous experiments using Rylo. Fractions of these dispersions were filtered through a 0.45 μm membrane filter

(filtration can easily be done by hand using a syringe) without any change in macroscopic appearance. The maximum particle size detected by LM was slightly reduced. LD+PIDS give the same results for the filtered and the unfiltered dispersions, and SAXS reflections cannot be detected in any dispersion.

Samples of the filtered and unfiltered fractions were autoclaved (121°C, 15(+5) minutes). In the filtered and the unfiltered cases, milky white dispersions were obtained with macroscopically visible particles. As in the case of the non-autoclaved dispersions, no clear differences can be detected between the filtered and the not filtered dispersions after autoclaving.

Example 5 - Effect of Heat Treatment Time

A dispersion containing Myverol 18-99 as monoolein and 12% poloxamer was divided into four fractions. Three fractions were autoclaved at 121°C for different periods of time (5 min, 15 min (+5 min), 30 min (+5 min)) and compared to the fourth, non-autoclaved fraction. During autoclaving, the opaque dispersion turned to milky white and visible aggregates appeared. In SAXS, the autoclaved dispersions display diffraction patterns according to the cubic P phase. In the case of the non-autoclaved dispersion no reflections can be detected, not even by the use of synchrotron radiation. LD+PIDS give monomodal particle size distributions for all dispersions, with a mode at ca. 360 to 390 nm for the autoclaved dispersions and a mode at ca. 88 nm for the non-autoclaved dispersion (Fig. 5). There are no detectable differences by any applied method between the autoclaved dispersions. Autoclaving time has thus no significant effect on the properties of the resulting dispersions in the range from 5 to 30(+5) minutes at

this temperature.

Example 6 - Influence of Temperature

A dispersion containing Dimodan DGMO as monoolein was divided into four fractions. Two fractions were heated to 80°C for different periods of time (20 min and 60 min), one fraction was autoclaved (121°C / 15(+5) min) and one fraction was left unchanged. Autoclaving changed the dispersion from opaque to milky white, heating to 80°C led to nearly milky white dispersions (very slightly opaque) in both cases. The LD+PIDS results indicate that the particle size distributions slightly shifted to larger particles during heating to 80°C (Fig. 6); there is no difference between the two 80°C-dispersions (20 min and 60 min). A second dispersion with Dimodan from a different container (container 2, same batch) showed nearly the same particle size distribution in the unheated case (the small peak at about 0.35 μm in the dispersion from container 1 is the averaging result of a bigger peak in one measurement run of five, the other runs showed the same particle size distribution as the dispersion from container 2), and increased particle sizes after autoclaving. Compared to autoclaving at 121°C, heating the dispersions to 80°C led to minor changes in particle size distribution (by means of LD+PIDS). In this case it therefore appears that temperatures higher than 80°C are necessary to form the large proportions of non-lamellar particles.

Example 7 - Influence of Monoolein Type

Autoclaving (121°C/15 min (+5 min)) dispersions

containing 12% Poloxamer with GMOrphic-80 or Myverol 18-99, respectively, as monoolein leads to particle size distributions in a similar range. Also the particle size distributions of the corresponding non-autoclaved dispersions are comparable with each other. Even though the use of Dimodan DGMO leads to similar non-autoclaved dispersions, autoclaving of these dispersions leads to different, smaller particle sizes.

Example 8 - SAXS Experiments

SAXS experiments on the dispersions of the previous examples were performed. Generally the unheated/non-autoclaved dispersions containing 12% poloxamer did not display X-ray reflections and only in a few cases were extremely weak, unassignable reflections observed. The heated dispersions (80°C: 20 min and 60 min) display very weak reflections due to cubic P phase. In the case of the autoclaved dispersions (121°C, 5 min, 15 min and 30 min), weak reflections for the Dimodan dispersions and clear reflections for the GMOrphic and Myverol dispersions were obtained, all pointing to cubic P phase.

Example 9 - Further influence of temperature

For further investigation of the influence of the temperature applied during the heating process after homogenization, a dispersion containing GMOrphic-80 as monoolein (MO) and 12% P407 (based on the sum of MO and P407) was prepared according to the standard procedure (Example 1). Fractions of the homogenized dispersion were heated to 90°C, 100°C, 110°C and 121°C, respectively, for 20 minutes, and compared to a non-heated fraction (Fig. 7).

With increasing temperature, the mean particle size increases and the PSD becomes narrower. There is only a weak difference in the results obtained after heating to 110°C and 121°C, which lead to the assumption that heating to higher temperatures than 121°C will probably not result in a narrower PSD. After heating to 90°C, ca. 50% of the particles were larger than 0.2 μm and clear SAXS reflections (cubic P) were observed, in contrast to the result after heating to 80°C (see Example 6), where 90% of the particles remained smaller than 0.2 μm and only very weak SAXS reflections (probably cubic P) were detected. The non-heated fraction and the 121°C/15(+5)min fraction give the usual results obtained earlier. It was concluded that in this case the minimum temperature necessary for PSD narrowing and conversion to non-lamellar particles was in the region of 90°C.

Example 10 - Influence of poloxamer concentration

For testing the influence of poloxamer 407 concentrations above 12% on the effect of autoclaving, dispersions containing 12%, 14% and 16% P407 were prepared according to the standard procedure. Fractions of these dispersions were autoclaved (121°C/15(+5) min) and compared to the non-autoclaved fractions (Fig. 8).

In both cases (autoclaved and non-autoclaved), no difference can be detected between the 12% dispersion and the dispersions with higher concentrations of P407 by visual inspection, light microscopy and SAXS. All of the non-autoclaved dispersions were opaque and displayed no SAXS reflections. After autoclaving, they turned into milky-white dispersions with large aggregates, and displayed clear SAXS reflections according to cubic P

with nearly the same lattice constants.

The LD+PIDS results demonstrate that increasing the P407-concentration from 12% to 14% slightly reduces the fraction of particles in the 0.2 - 0.5 μm range in the non-autoclaved dispersions. Further increasing of the P407-concentration had no effect on the LD+PIDS result. The mode value and the width of the PSD for the autoclaved dispersion are slightly different for the different P407-concentrations despite the fact that they were autoclaved together by the same autoclaving process. No correlation was seen between P407-concentration and PSD mode value or PSD width.

Example 11 Influence of poloxamer type

To test the influence of the poloxamer type on the properties of the resulting dispersions, poloxamer 188 (P188) was used instead of P407. A dispersion was prepared according to the standard procedure (Example 1) with P188-concentrations of 8.75 weight-% (based on the sum of MO and P188). This concentration of P188 is equivalent (when calculated as mol-%) to the usual concentrations of P407 (12 weight-%). Fractions of this dispersion were autoclaved (121°C/15(+5) min). The dispersion was compared to a non-autoclaved and autoclaved dispersion with 12% P407 (Fig. 9).

The homogenized (non-autoclaved) dispersion with 8.75% P188 was homogenous and nearly milky white. SAXS reflections were not detected and LD+PIDS displayed a PSD with a slightly higher amount of particles in the size range of ca. 0.2 - 0.5 μm compared to the non-autoclaved dispersion with 12% P407. The autoclaved fraction of this dispersion was milky-white with large

aggregates and displayed clear cubic P SAXS reflections, like the autoclaved dispersion with 12% P407 do (see Fig. 10). A very weak peak in the autoclaved 8.75% P188-dispersion between the first and the second cubic P reflection is in the region where the first reflection of a cubic D phase would be expected and may indicate a small amount of cubic D phase in this dispersion. The lattice constant (of the cubic P phase) is smaller in the case of the dispersion containing 8.75% P188 (ca. 13.5 nm) compared to that of the dispersion containing 12% P407 (ca. 14.4 nm). The PSD (LD+PIDS) was nearly the same as that of the autoclaved dispersion with 12% P407.

Example 12 - Influence of long-term storage

To answer the question, whether the lamellar particles of a non-autoclaved dispersion with 12% P407 transform into non-lamellar particles with time without heat treatment, or whether the cubic particles produced by autoclaving a dispersion with 12% P407 transform back to lamellar particles with time, dispersions (12% P407, non-autoclaved and autoclaved) were investigated by SAXS after a storage period of 6 months (at 23°C, called "stored dispersions") after preparation. The results were compared to the SAXS results of these dispersions obtained 20 days (stored at 23°C, called "unstored dispersions") after preparation (Fig. 11).

In the case of the autoclaved dispersion, the difractograms of both dispersions (stored and unstored) display clear cubic P reflections, the lattice constants are the same (14.4 nm). No additional reflections occur after storage (a phase change to cubic D or hexagonal with time, possibly caused by, e.g., hydrolysis of the monoolein, would result in additional reflections).

In the case of the non-autoclaved dispersion, there are no reflections detectable in the difractograms of either system. The result, that no detectable cubic P phase is formed in non-autoclaved dispersions (with 12% P407) by time, was confirmed by examination of a second, independent batch (after 7 days and 6 months after preparation).

Example 13 - Influence of Drug Loading

Five different drugs (ubidecarenone, tocopherol acetate, miconazole, betamethasone-17-valerate, chloramphenicol) were incorporated in a monoolein (GMOrphic) dispersion stabilized with 12 % P407 (which forms a lamellar vesicular dispersion in the unloaded state) by adding the drugs to the MO/P407 melt at 60°C (or 80°C for concentrations of 5 % drug) in the "standard" preparation process (see Example 1). All drug concentrations are indicated relative to the sum of monoglyceride and poloxamer. A drug-free dispersion was prepared and investigated as a reference.

All dispersions were autoclaved at 121°C for 15 + 5 min. (allowing for temperature equilibration in the autoclave) and their properties were compared to that of the corresponding non-autoclaved dispersions.

Ubidecarenone and tocopherol acetate at a concentration of 0.3 % did not influence the properties of the resulting dispersions. The transformation of lamellar vesicular into non-lamellar (cubic) particles upon autoclaving proceeded as in the drug-free dispersions. Higher concentrations of these drugs were not investigated.

Dispersions with 0.3, 1 or 2 % betamethasone-17-valerate also had no influence on the general behaviour of the dispersions. A drug load of 5 % could not be realized with this substance since it could not be dissolved in the MO/P407 melt at this concentration.

Chloramphenicol at 0.3, 1 and 2 % as well as miconazole at 0.3 and 1 % had no influence on the non-autoclaved dispersions. In autoclaved dispersions, however, a concentration dependent influence could be observed: In chloramphenicol-loaded dispersions the particle sizes increased distinctly with drug concentration and a slight increase in lattice constant of the cubic phase was observed. 5 % chloramphenicol could be incorporated in the MO-dispersion but homogenization as well as autoclaving led to dispersions with distinctly larger particle sizes in comparison to the drug free dispersions and those with up to 2 % drug.

For the 5 % chloramphenicol sample, cubic reflections could be observed in small angle X-ray scattering even before autoclaving. The lattice constant of the cubic phase in the (non-autoclaved and autoclaved) 5 % sample is much larger than in the autoclaved drug-free dispersion or (autoclaved) dispersions with up to 2 % chloramphenicol.

Miconazole could be incorporated at concentrations of 0.3 and 1%. Homogenization of these dispersions led to opaque dispersions without cubic X-ray reflections in all cases. Autoclaving led to slightly larger (0.3 %) and distinctly larger (1 %) particle sizes compared to the dispersions without drug incorporation. The lattice constant decreased slightly.

Example 14 Autoclaving of a liposomal dispersion

In order to assess whether a standard liposomal dispersion having a lamellar equilibrium form at room temperature would convert to non-lamellar particles under heating, the method was tested on a liposomal dispersion.

To prepare the liposomal dispersion, 5 % egg phospholipid (Lipoid E80) was stirred in water (containing 0.01 % thiomersal as a preservative) for one day at room temperature and subsequently extruded (Avestin Emulsiflex-C5) 10 times through a 100 nm polycarbonate filter. The resulting dispersion had a PCS z-average diameter of 117 nm with a polydispersity index of 0.08.

One fraction of the dispersion was autoclaved for 15 + 5 min. at 121°C and the properties of the resulting dispersion were compared to that of the non-autoclaved one. Except for slight differences in optical appearance no differences between the two samples were observed with the following methods:

Both samples are visually homogenous without macroscopically detectable particles and of yellowish-opaque appearance with a slightly more intense colour after autoclaving. The particle size measurement with laser diffraction + PIDS yields a monomodal particle size distribution with a mode at 106 nm for both dispersions (Fig. 12). Both dispersions display diffuse small angle X-ray scattering without detectable sharp reflections, indicating the presence of only lamellar particles (Fig. 13).

Example 15 - Compositions including fatty acids

Pre-formulations were prepared using the standard method indicated in Example 1 but including the fatty acid oleic acid in the formulation.

a) An initial melt was prepared containing GMO (85.5%), oleic acid (4.5%) and Lutrol F127 (10%). To 9g of water under mechanical stirring was added 1g of the molten mixture to form a coarse dispersion. This was examined for phase structure and comprised principally cubic liquid crystalline phase particles of average diameter greater than 100 μm. The particle size distribution of the coarse dispersion is shown as "a" in Figures 14 and 15.

b) The coarse dispersion was divided into two portions. The first portion was homogenised with the microfluidiser at 345 bar and the second portion homogenised with the microfluidiser at 172 bar. The particle size distributions of the two resulting dispersions are indicated as "b" in Figures 14 and 15 respectively. It can be seen that higher pressure homogenisation gave a mono-modal particle size distrubution of relatively small particles and lower pressure homogenisation gave larger particles with a bimodal distribution.

c) The two dispersions prepared in part ii were each heated to 120°C for 20 minutes and the particle phase and size distributions reexamined. The results indicated largely cubic liquid crystal particles with size distributions as indicated as "c" in Figures 14 and 15. The maximum particle sizes remained essentially static but the width of the distribution decreased notably in the case of the low pressure homogenisation (Figure 15) and remarkably in the case of the high pressure homogenisation (Figure 14).

After heat cycling, both compositions were of colloidal particles and had sharp, narrow particle size distributions. Such dispersions are thus highly suitable for both intravenous administration and controlled release applications by any suitable administration route.

Example 16 - Storage stability

The dispersions prepared in Example 15 parts (b) (before heat treatment) and (c) (after heat treatment) resulting from high pressure homogenisation were stored for 11 days at room temperature. After storage the particle size distribution was again examined and is indicated in Figure 16.

The effects of storage on particle size may be seen by comparing Figures 14 and 16. It can be seen that the non-heat treated sample ("a" in Figure 16) increased somewhat in mode particle size and showed a bimodal distribution after storage, with a secondary portion of particles above 1 μm in diameter. In contrast, the heat treated sample ("b" in Figure 16) shows a distribution of particles indistinguishable from that prior to storage ("c" in Figure 14). Thus, the heat treatment cycle not only narrowed the particle size distribution of the sample but also rendered the sample more stable to storage.

Claims

1. A method for the production of (preferably colloidal) non-lamellar particles, said method comprising forming lamellar and optionally non-lamellar particles comprising at least one structuring agent, heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide conversion of at least 50% of said lamellar particles to non-lamellar form, after cooling.

2. A method for narrowing the particle size distribution of a sample of lamellar and/or non-lamellar particles comprising at least one structuring agent, said method comprising heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide a narrowing of said particle size distribution, after cooling.

3. A method for stabilising the particle size distribution (for example, as displayed by light scattering) of a sample of lamellar and/or non-lamellar particles comprising at least one structuring agent, said method comprising heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide stabilisation of said particle size distribution after cooling.

4. Non-lamellar particles comprising at least one

structuring agent formed or formable by forming lamellar and optionally non-lamellar particles comprising at least one structuring agent, heating said particles to an elevated temperature, followed by cooling, preferably to ambient temperature, wherein said heating is to a temperature and for a period sufficient to provide conversion of at least 50% of said lamellar particles to non-lamellar form, after cooling.

5. A formulation of (preferably colloidal) particles comprising at least one structuring agent, wherein at least 75% of the particles, preferably at least 85% and most preferably at least 95% of particles in the formulation are non-lamellar.

6. A formulation as claimed in claim 5 further comprising at least one active agent.

7. A formulation of particles as claimed in claim 5 or claim 6 wherein the particle size distribution is essentially stable to storage at room temperature for at least 10 days.

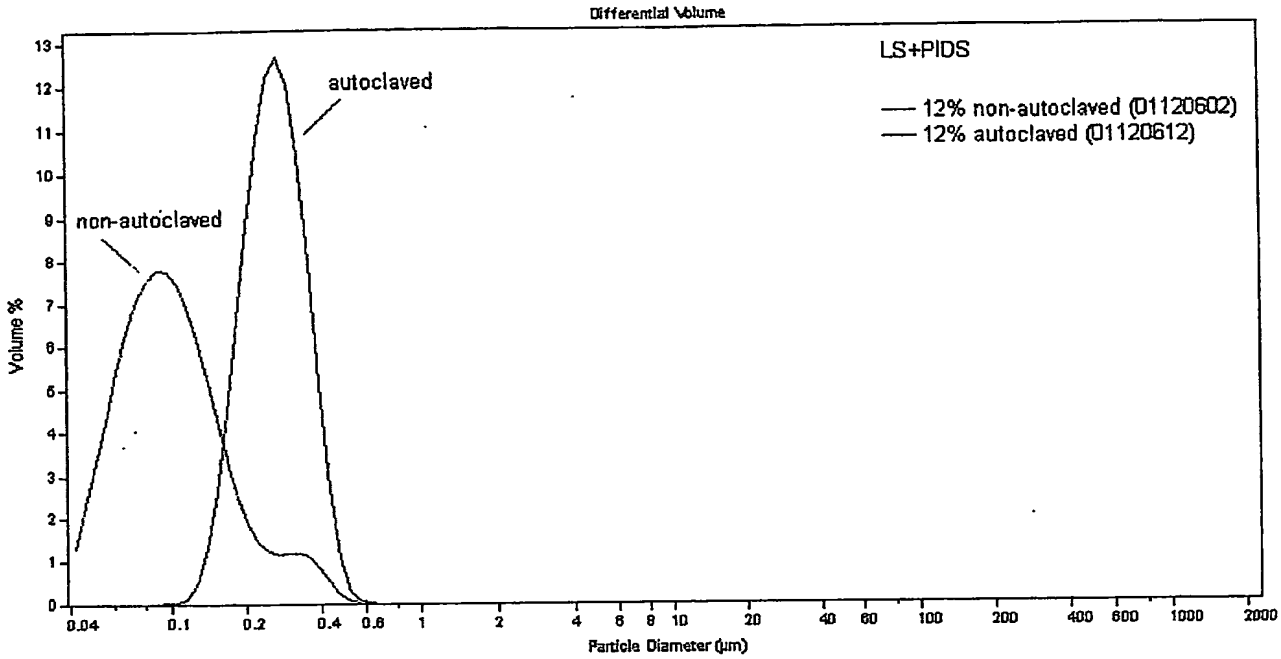


Figure 1

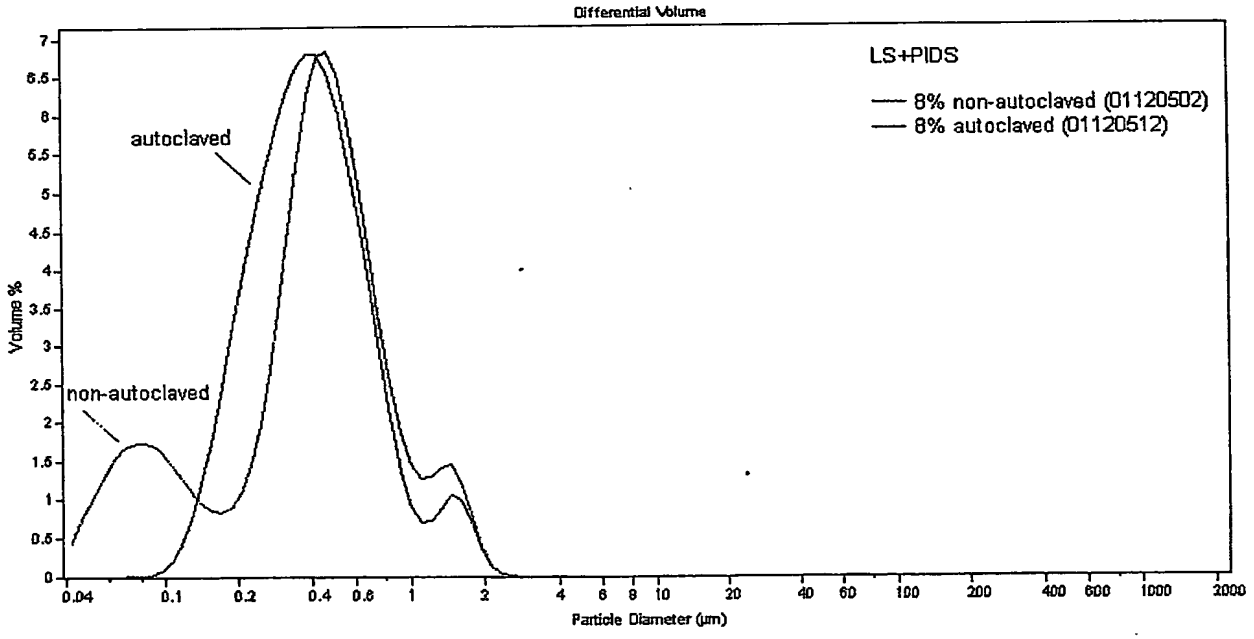


Figure 2

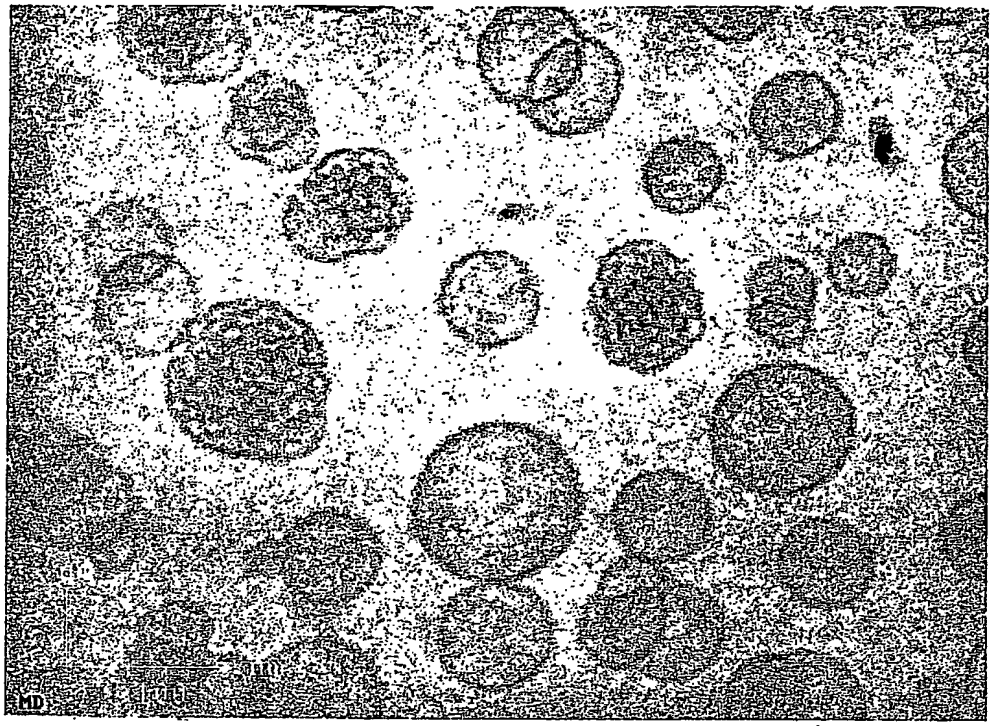


Figure 3

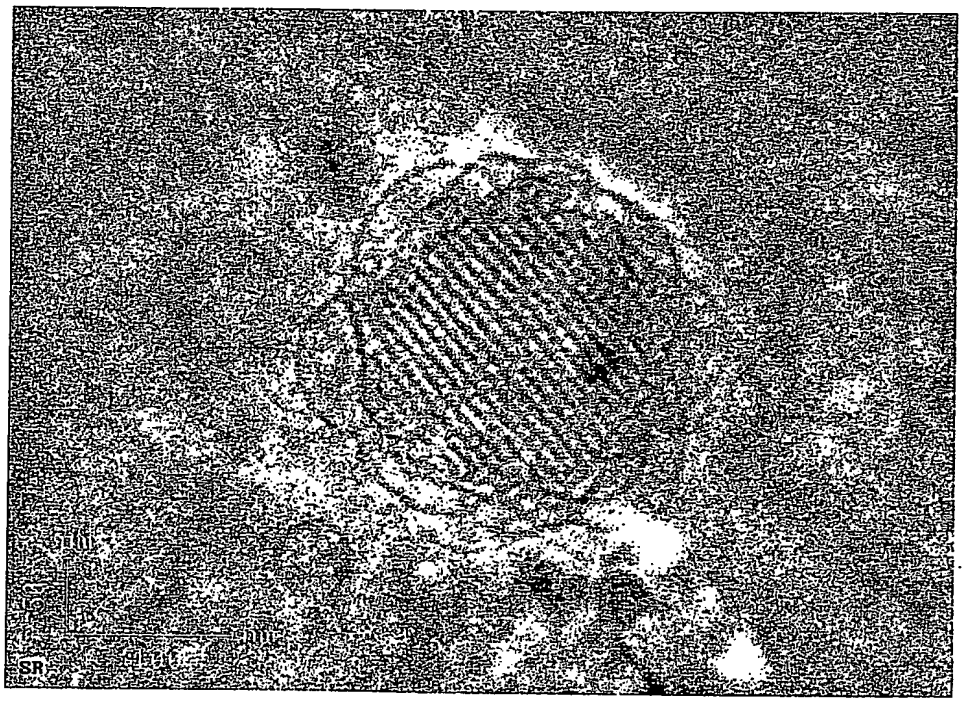


Figure 4

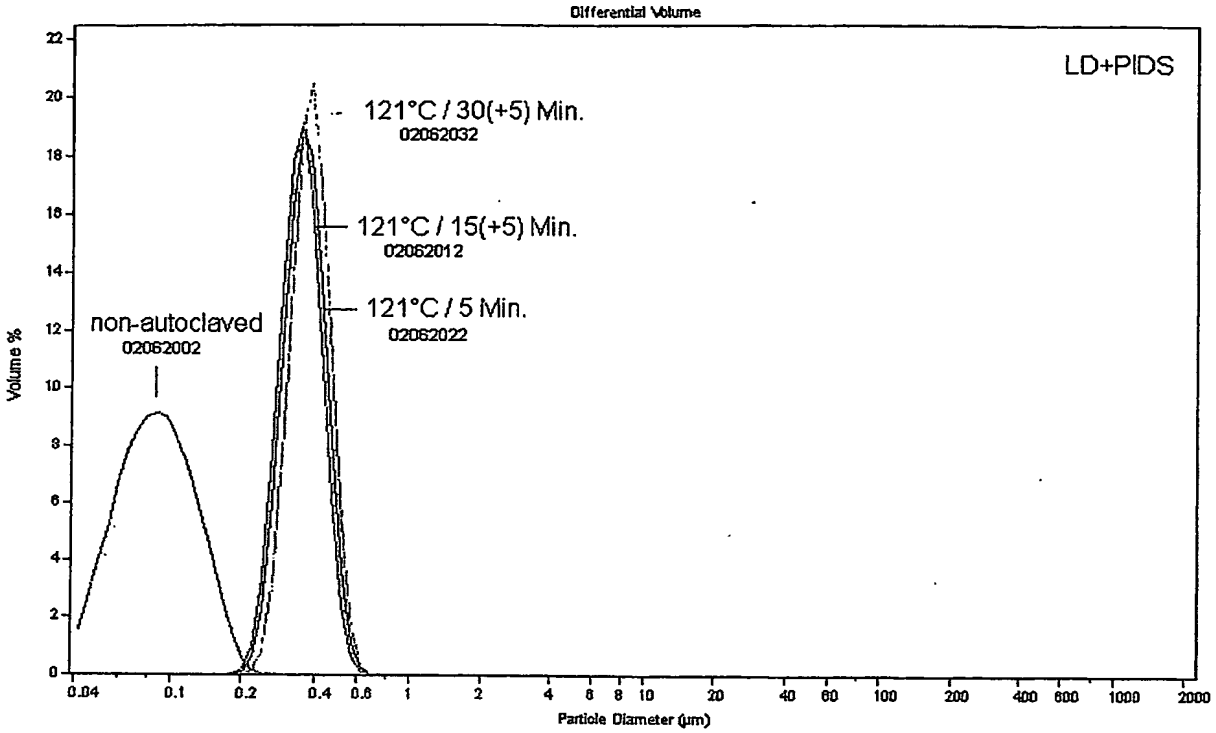


Figure 5

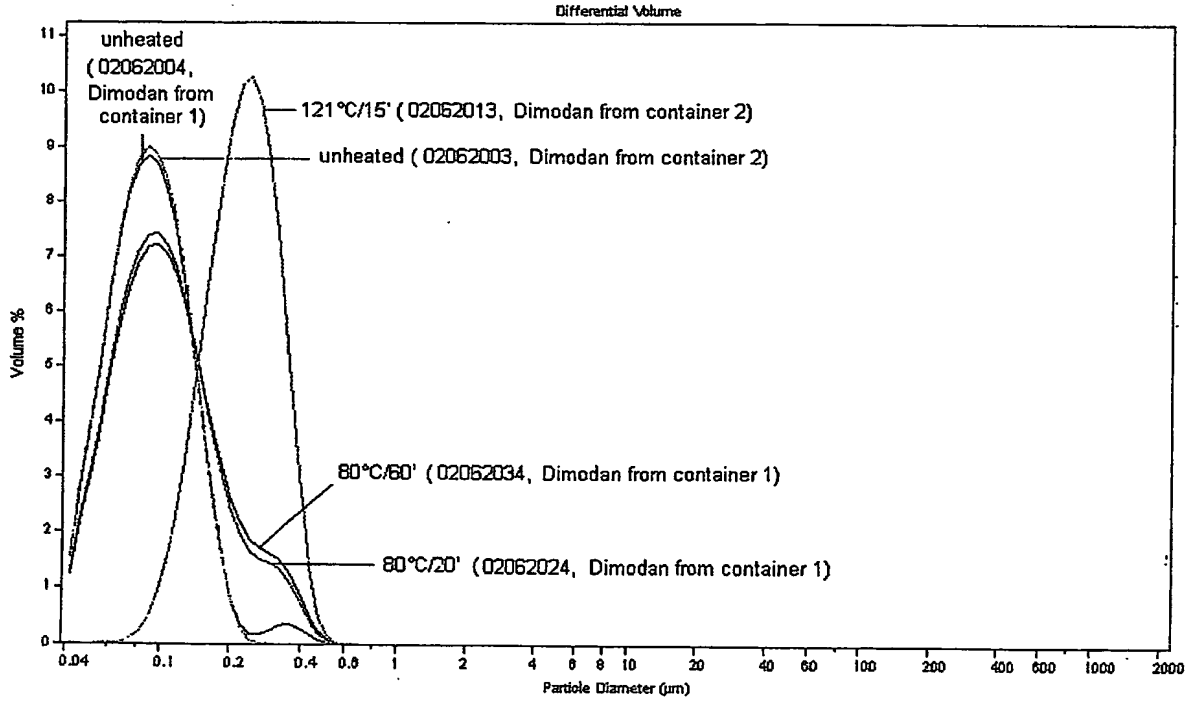
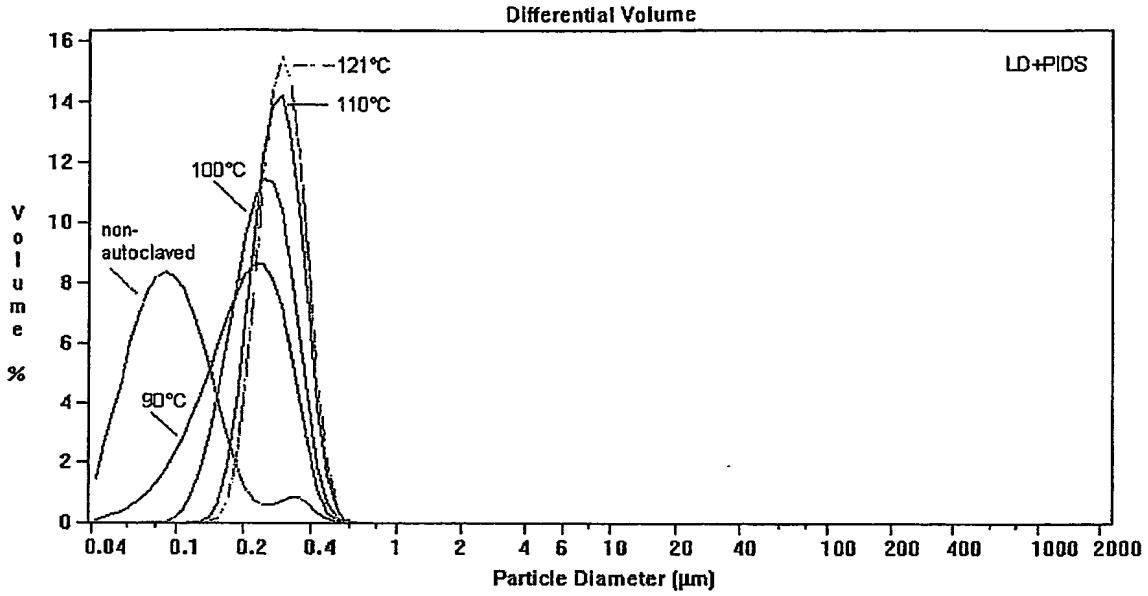


Figure 6



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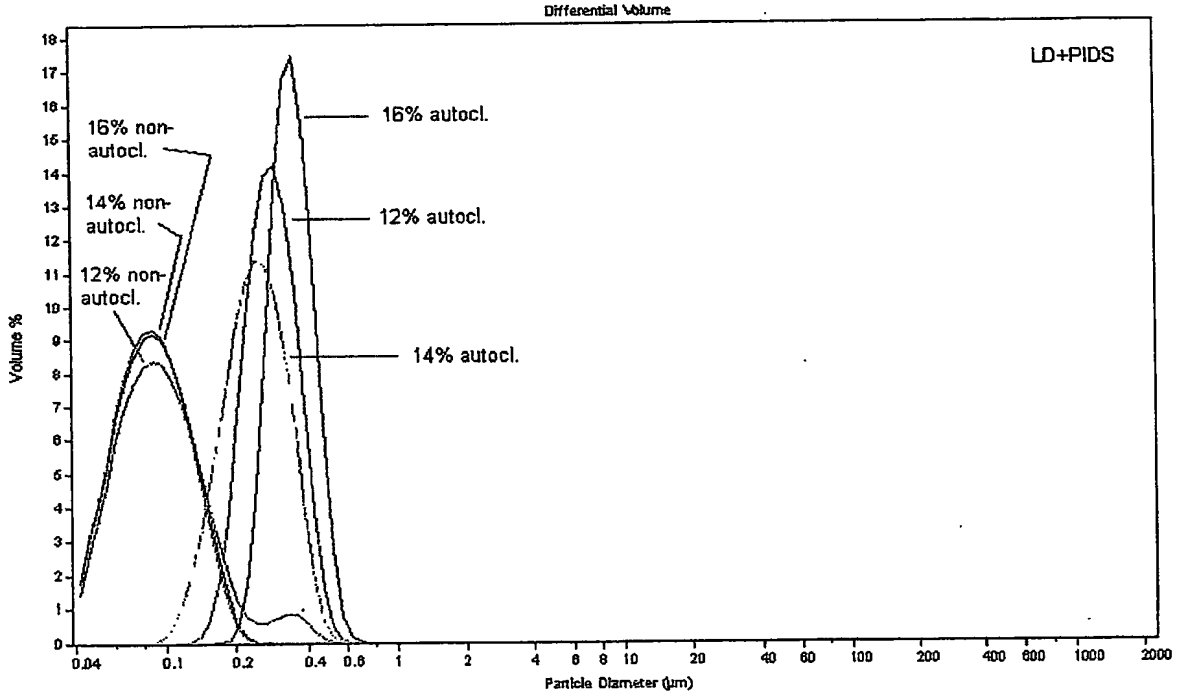

Figure 8

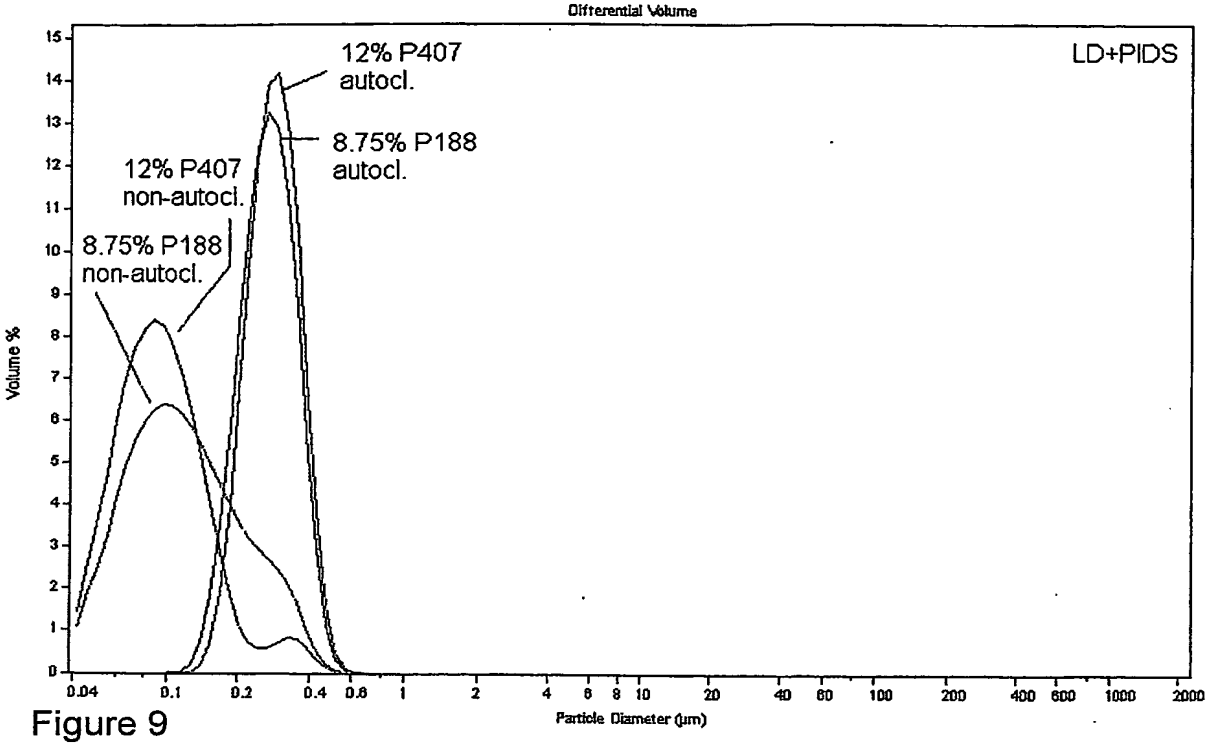

Figure 9

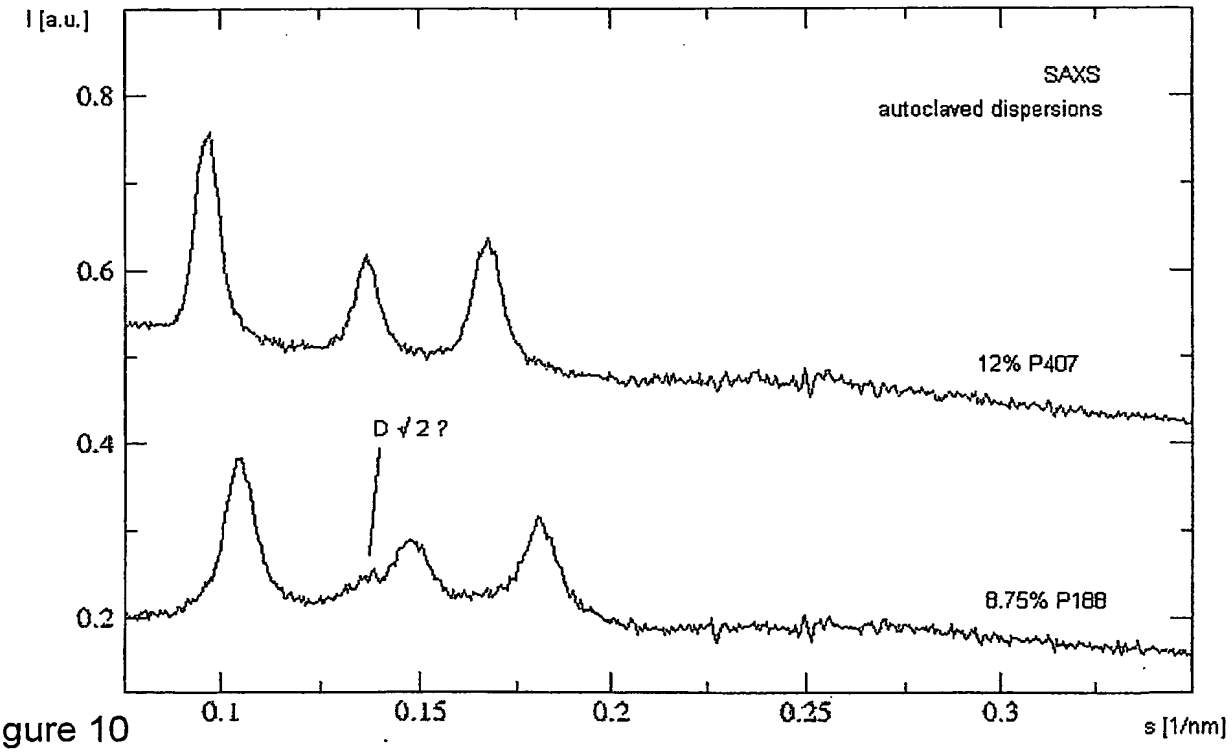

Figure 10

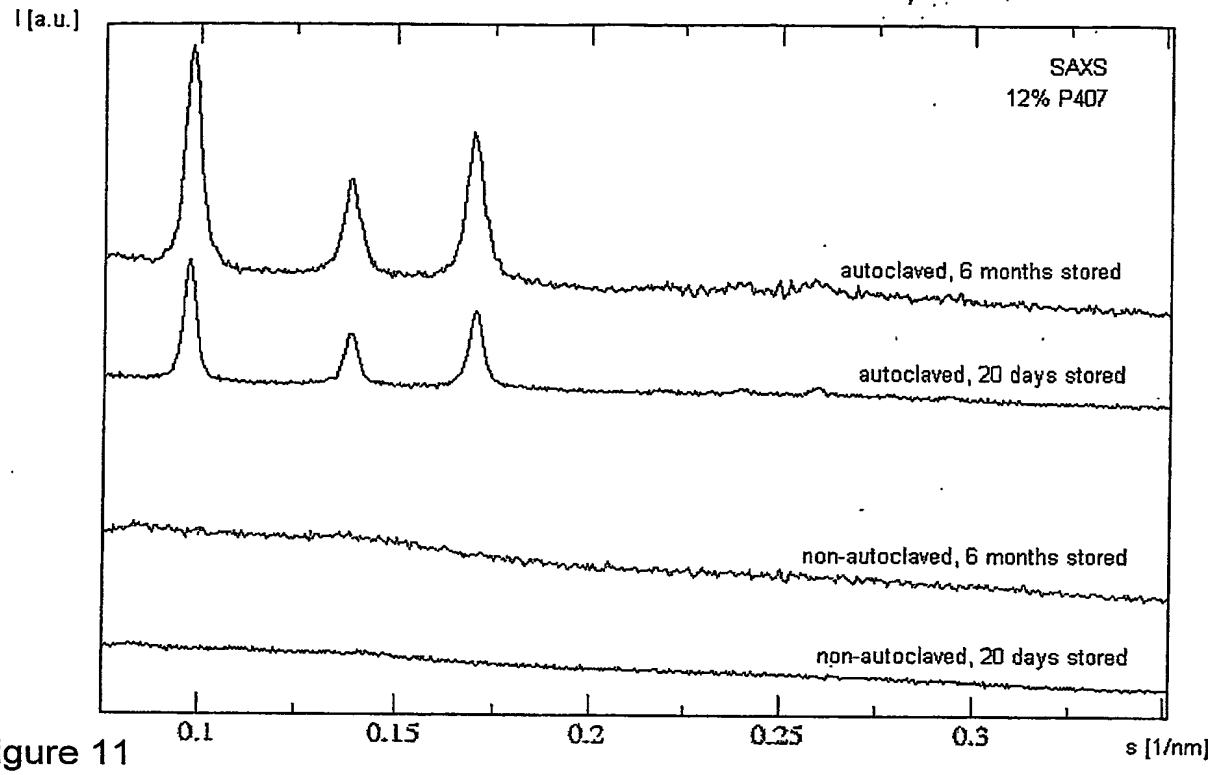

Figure 11

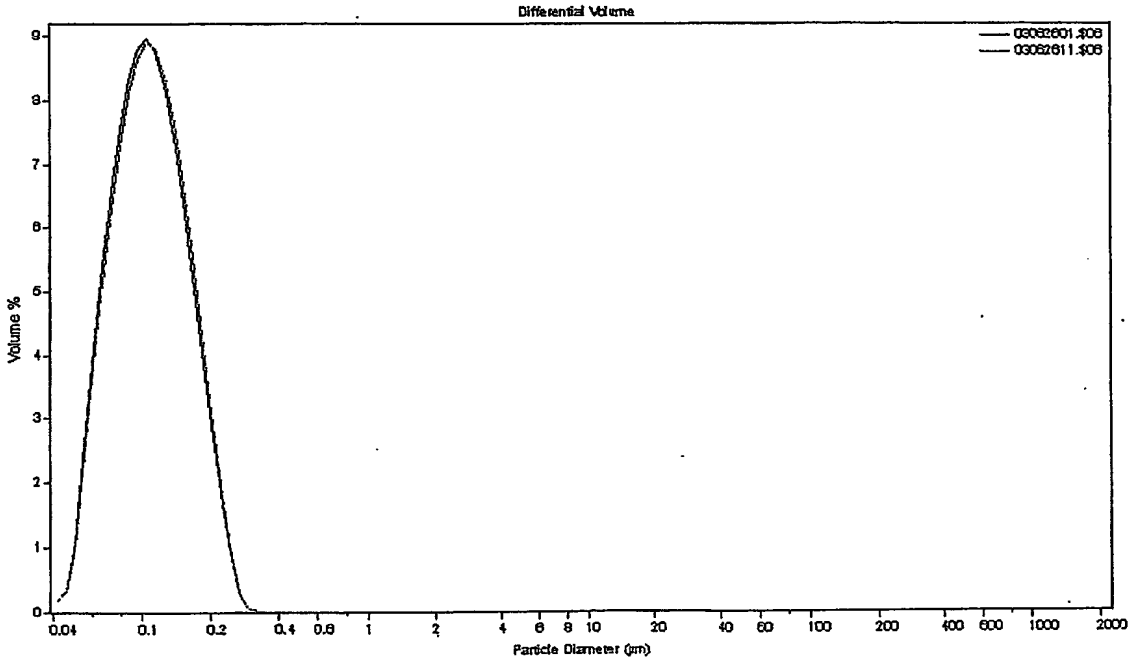

Figure 12

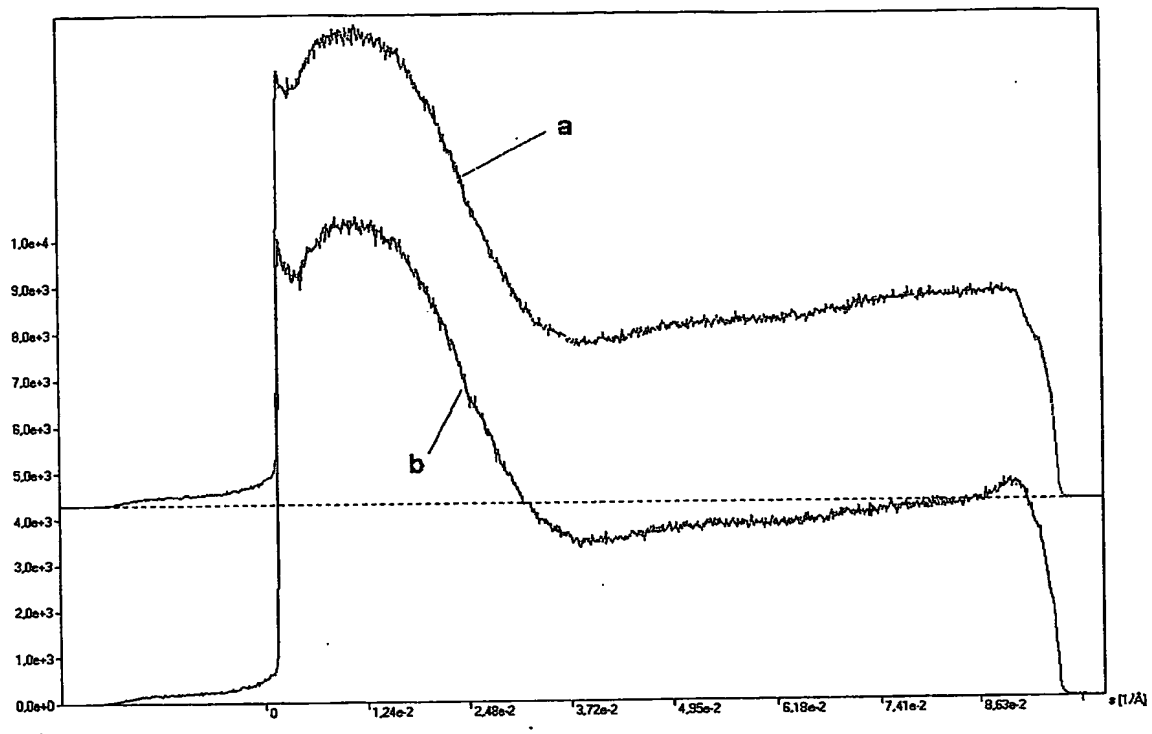

Figure 13

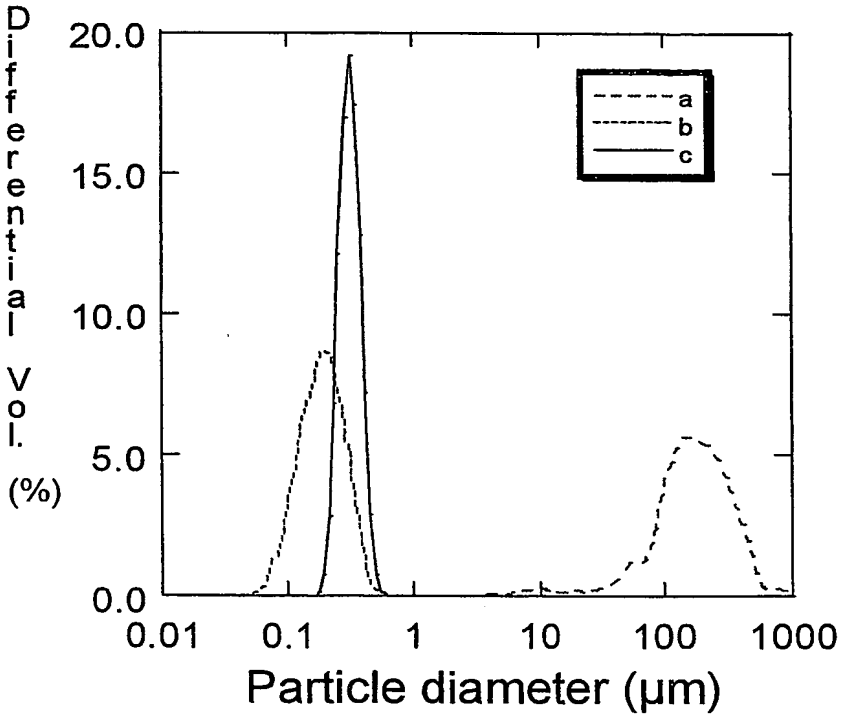

Figure 14. Particle-size distribution of liquid crystalline (cubic phase) dispersion after a) mechanical agitation, b) homogenisation by Microfluidizer (six passes) operating at 345 bar, and following c) heating to 120° C for 20 minutes.

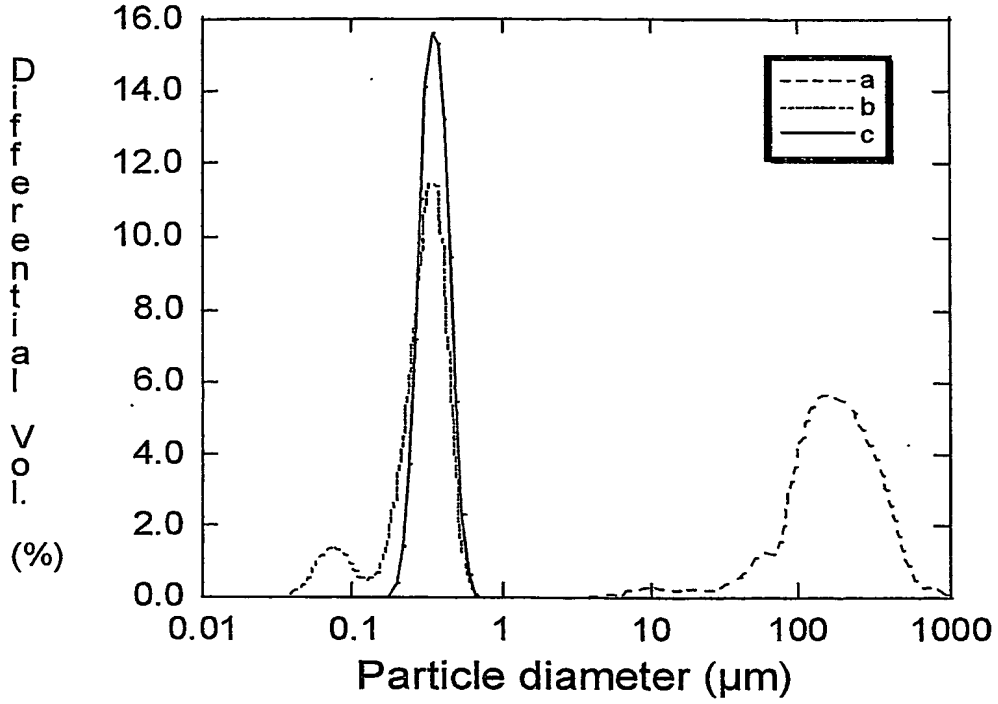

Figure 15. Particle-size distribution of liquid crystalline (cubic phase) dispersion after a) mechanical agitation, b) homogenisation by Microfluidizer (six passes) operating at 172 bar, and following c) autoclaving 120 C for 20 minutes.

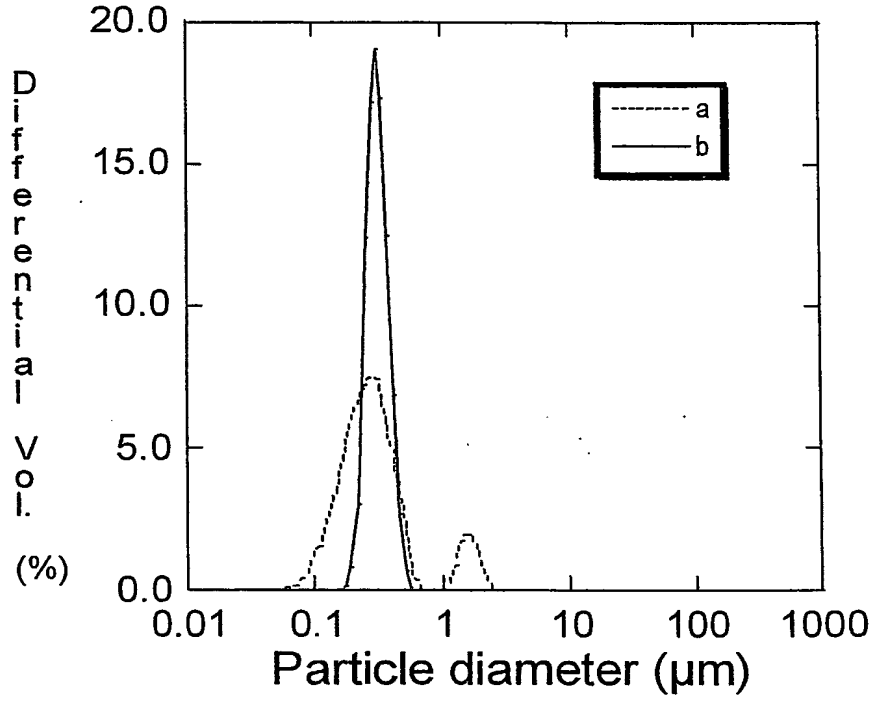

Figure 16. Particle-size distribution of liquid crystalline (cubic phase) dispersion after 11 days storage of sample a) homogenised by Microfluidizer (six passes) operating at 345 bar, and following b) heated to 120 C for 20 minutes.